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(54) Title: **TISSUE MARKING USING BIOCOMPATIBLE MICROPARTICLES**

(57) Abstract: Described are methods of tissue marking using microparticles that have carbon surfaces, and that contain a contrast agent. Preferred microparticles include a permanently radiopaque particle substrate and a pyrolytic carbon surface.

TISSUE MARKING USING BIOCOMPATIBLE MICROPARTICLES

Background

Tissue marking is a method of marking a site or position in a body to allow re-visiting of the position at a later time to check for progress or developments of an ailment or a treatment or to allow re-treatment at the same site. Tissue marking may be used during biopsy or other tissue-removal procedures to accurately mark the site of the tissue-removal or biopsy in order to allow a treatment-giver to later return to the same site if desired.

Such tissue marking may be useful in procedures relating to colon or rectum biopsies or tissue removal, prostate biopsies, or breast biopsies. Specifically with respect to breast biopsies, it is not uncommon in modern breast biopsies for all evidence of a lesion to be removed during biopsy. Removing all trace of the tissue also removes identifying features from the site and makes it difficult to return to the same location later to re-check the site. This dilemma, created by a removal of a potentially malignant breast mass or cluster of microcalcifications during core biopsy, can be ameliorated by placing radiographically visible markers immediately after the biopsy. The marker can be used to help locate the biopsy site in case malignancy is determined even if the mammographic findings associated with the original lesions were removed completely.

One localization or marking method involves placing a metallic clip (e.g., a clip sold under the trade name Micromark™, from Biopsys Medical) through an 11- or 14-gauge probe of a biopsy device and attaching the clip to the site of a biopsy to mark the location of the biopsy. These clips are approximately 3mm across and are permanent and radiopaque. The use of marking clips has been described by Burbank et. al., "Tissue Marking Clip for Stereotactic Breast Biopsy: Initial Placement Accuracy, Long-term Stability, and Usefulness as a Guide for Wire Localization," Radiology 1997; 205:407-415; and Liberman et. al., "Clip Placement After Stereotactic Vacuum-Assisted Breast Biopsy," Radiology, 1997; 205:417-422.

Another example of an application for tissue marking is in prostate biopsies. It is recognized that initial biopsies may not be fully determinative in the prostate. See Jonathan I., "Are You Getting Maximum Diagnostic and Prognostic Information from your Prostate Needle Biopsy?" Contemporary Urology, 106, April 1999. Tissue

marking can ensure that the tissue of non-determinative initial biopsies can be monitored for progressive disease and that, if necessary, a follow-up biopsy can be performed at the site of the initial biopsy.

Other methods of tissue marking or localization are described in Fajardo et. al.,
5 "Placement of Endovascular Embolization Microcoils to Localize the Site of Breast Lesions Removal at Sterotactic Core Biopsy," Radiology, 1998, 206: 275-278, Goldberg et. al., "Preoperative Localization of Non-Palpable Breast Lesions Using a Wire Marker and Perforated Mammographic Grid," Radiology 146: 833-835, March 1983 and United States Patents 4,341,220 and 5,665,092.

Summary of the Invention

The invention provides a method of tissue marking. The method includes the use of detectable, preferably radiopaque particles delivered to a tissue site for later detection. The particles can preferably be delivered into the body to a desired site by
15 injection using a hypodermic needle and syringe, or another similar instrument, or percutaneously with the assistance of a biopsy probe. Microparticles can preferably be of an average size in the range from about 100 to 1000 microns, more preferably from about 200 to 500 microns, and most preferably from about 251 to about 300 microns, in transverse, cross-sectional dimension. The microparticles can preferably be
20 permanently radiopaque and may optionally comprise a carbon coating.

An optional carbon surface can be coated onto a particle substrate as a thin coating or film, thereby creating a particle having a highly biocompatible carbon surface. While not required, pyrolytic carbon may be preferred.

The particle substrate can be, but is not necessarily, biocompatible and should
25 be capable of withstanding the conditions of a process for coating a carbon surface onto the substrate, which might include elevated temperatures. In particularly preferred embodiments, particle substrates can be radiopaque, most preferably permanently radiopaque. Exemplary radiopaque materials can include metals and metal oxides such as zirconium oxide and aluminum oxide, gold, titanium, silver,
30 stainless steel, oxides and alloys thereof.

The microparticles can be delivered using a fluid carrier which can be any biologically compatible material capable of delivering the microparticles to a desired

tissue site, such as a biologically compatible suspension, solution, or other form of a fluid or gel. Examples of materials useful in biologically compatible carriers include saline, dextrans, glycerol, polyethylene glycol, corn oil or safflower, other polysaccharides or biocompatible polymers, methyl cellulose, glucan, agarose, either
5 singly or in combination.

The use of microparticles in tissue marking methods, preferably by injecting through a hypodermic needle and syringe or a like instrument, has advantages over other tissue marking methods. For instance, delivery of microparticles using a needle and syringe allows very precise delivery of microparticle markers to a desired tissue
10 site; this is particularly true if a biopsy probe used to perform a biopsy is used to assist delivery of microparticles for tissue marking, without first moving the biopsy sheath. Additionally, microparticles can be used in tissue locations where other types of tissue markers are not or cannot be used. For example, some tissue locations such as the colon or rectum do not lend themselves to the use of marking clips, yet it is possible to
15 deliver microparticles to these locations for effective marking. Further, preferred embodiments of the microparticles can be permanently radiopaque. The location of permanently radiopaque particles can be monitored, by known methods, for as long as the radiopaque microparticles remain in a body.

For purposes of the present disclosure, the following terms shall be given the
20 following meanings.

The term "biocompatible," refers to materials which, in the amount employed, are non-toxic and substantially non-immunogenic when used internally in a patient and which are substantially insoluble in blood. Suitable biocompatible materials include ceramics, metals and metal oxides such as titanium, gold, silver, stainless steel, oxides
25 thereof, aluminum oxide, zirconium oxide or carbon such as pyrolytic, low temperature or ultra low temperature isotropic carbon.

The term "detectable" refers to materials capable of being detected during or after injection into a patient by methods generally used for monitoring and detecting such materials, such as magnetic resonance, X-ray, ultrasound, magnetotomography, electrical impedance imaging, light imaging (e.g. confocal microscopy and
30 fluorescence imaging) and nuclear imaging (e.g. scintigraphy, SPECT and PET). Examples include contrast-enhancing agents such as radiopaque materials. Contrast-

enhancing agents may be either water soluble or water insoluble. Examples of water soluble radiopaque materials include metrizamide, iopamidol, iothalamate sodium, iodomide sodium, and meglumine. Examples of water insoluble radiopaque materials include metals and metal oxides.

5

Detailed Description

The invention provides methods of marking tissue for any reason, such as to mark the site of the removal of a tissue (removal of a polyp from a colon or rectum); to mark the site of a biopsy (a breast biopsy, a prostate biopsy, a colon biopsy, or a rectum biopsy); or to mark the site of any other medical procedure. The tissue may be marked to return to the same tissue site to monitor the progress of a medical condition or a treatment, or to perform a subsequent biopsy at the same site. In addition, detectable microparticles can be delivered to a tissue site to act as a target at which or near a beam of radiation can be precisely directed.

The invention involves marking a tissue site using detectable microparticles, preferably that are also biocompatible. The microparticles contain some detectable component (some well-known detectable components are referred to as a "contrast-enhancing agent") that causes the microparticles to be tracked, monitored, or otherwise detected by known methods, including radiography or fluoroscopy. The detectable component can be any material capable of enhancing contrast in a desired imaging modality (e.g. magnetic resonance, X-ray, ultrasound, magnetotomography, electrical impedance imaging, light imaging (e.g. confocal microscopy and fluorescence imaging) and nuclear imaging (e.g. scintigraphy, SPECT and PET)). Contrast-enhancing agents are well known in the medical arts, with any of a variety of such contrast-enhancing agents being suitable for use according to the methods of the invention.

A detectable component is preferably capable of being substantially immobilized within a microparticle, and may be incorporated into a microparticle for use in tissue marking in any of a variety of ways, such as part of a particle substrate, as a surface coating, as an additive to a surface coating such as a carbon coating, or elsewhere. A detectable component can be added to a material that is not radiopaque.

The detectable component may be provided in any location or portion of a microparticle by known methods.

According to an embodiment of the invention, the microparticles comprise a permanently radiopaque material which can be detected within a body following delivery to a tissue site. Permanent radiopacity is unlike contrast-enhancing agents or radiopaque materials which biodegrade or otherwise lose their effectiveness (detectability or radiopacity) over a period of days or weeks, such as 7 to 14 days. An advantage of permanent radiopaque materials is that they can be detected for as long as they remain in a body, whereas non-permanent radiopaque materials or other types of contrast-enhancing agents are detectable for only a limited time. See copending United States Patent Application Serial Number 09/602,323, entitled Embolization Using Carbon Coated Microparticles, and filed on June 23, 2000.

Some examples of radiopaque materials include paramagnetic materials (e.g. persistent free radicals) and compounds, salts, and complexes of paramagnetic metals (for example transition metal or lanthanide ions); heavy atom compounds (i.e. atomic number of 37 or more) salts, or complexes (e.g. heavy metal compounds, iodinated compounds, etc.); radionuclide-containing compounds, salts, or complexes (e.g. salts, compounds or complexes of radioactive metal isotopes or radiodinated organic compounds); and superparamagnetic materials (e.g. metal oxide or mixed oxide particles, particularly iron oxides). Paramagnetic metals include Gd (III), Dy (III), Fe (II), Fe (III), Mn (III) and Ho (III), and paramagnetic Ni, Co and Eu. Heavy metals include Pb, Ba, Ag, Au, W, Cu, Bi and lanthanides such as Gd.

The amount of detectable material included in a microparticle used for tissue marking should be sufficient to allow detection of the microparticle as desired. The amount used in any particular application or microparticle may depend on various factors such as the size of the microparticles, the total amount of microparticles delivered, the type of contrast-enhancing agent, etc. According to some embodiments of the invention, microparticles can be made up of 100 percent radiopaque material. Alternatively, for radiopaque particles that are coated with a carbon surface, the microparticles can have any relative amounts of radiopaque particle substrate to carbon coating that will allow the microparticles to be used as detectable tissue markers, for example from about 50 to 100 percent by weight radiopaque particle substrate based

on the total weight of the particle substrate and the carbon coating. Optionally, some, i.e., only a portion, but not all microparticles used in a particular tissue marking procedure (tissue marking composition, as described below) can include a detectable component.

5 In one embodiment of the invention, the microparticles are completely made up of permanently radiopaque material, in a form that is biologically compatible, and are delivered directly to a tissue site as such.

In another embodiment, microparticles for tissue marking according to the invention can have a surface that comprises carbon. The carbon-containing particle
10 surface may be in the form of a carbon-containing coating or carbon-containing film of any type of carbon, e.g., pyrolytic carbon such as low temperature isotropic (LTI) carbon, another type of isotropic carbon, or vitreous carbon, preferably in a form that is biocompatible. Various forms of carbon are described in Beavan, "Material Properties and Applications of Pyrolite® Carbon," Materials Engineering, February 1990.

15 Examples of carbon coated particles are described in United States Patent 5,792,478.

The atomic structure of both pyrolytic carbon and vitreous carbon is similar to graphite, a common form of carbon, but the alignment between hexagonal planes of atoms is not as well ordered as in graphite. Pyrolytic carbon is characterized by a more
20 chaotic atomic structure with warped hexagonal planes, missing atoms, and generally a more turbostatic appearance. This results in better bonding between layer planes.

The carbon coated microparticles can be constructed as a particle substrate having a carbon surface. While the substrate need not be biocompatible due to its being coated with a biocompatible layer comprising carbon, it can be preferred that the
particle substrate may also be biocompatible.

25 Such carbon coated microparticles may be prepared using any of a variety of coating processes to deposit carbon onto a particle substrate. A particle substrate can be selected for compatibility with the coating process, meaning that it should be capable of withstanding temperatures used in a given process for coating carbon onto a particle substrate. Relatively hard metallic or ceramic materials capable of
30 withstanding high temperature conditions of a coating process are generally preferred materials for a particle substrate. Metals, metal oxides, and alloys, including but not limited to medical grade stainless steel, silver, gold, titanium and titanium alloys, and

oxide derivatives of stainless steel or titanium or titanium alloys, are also acceptable materials for the particle substrate, with aluminum oxide and zirconium oxide being especially suitable. Carbon itself in any of its various forms, such as pyrolytic carbon, non-pyrolytic carbon, isotropic carbon, graphite, or vitreous carbon, may be useful as a particle substrate material. Thus, the microparticles may include a carbon coating deposited on a carbon particle substrate, and may be substantially or entirely made of carbon. In one embodiment of the invention, both the particle substrate and the carbon coating may comprise pyrolytic carbon.

Particle substrates intended to be coated with carbon, whatever their composition, should be of sufficient diameter, shape, and uniformity that they can be coated with carbon to produce carbon coated particles of a size, quality, and nature as described herein. Preferably, the particle substrates, prior to coating, can be selected and milled, extruded, sifted, cleaned, filtered, or otherwise formed to provide a desired combination of particle size, shape, and quality, to result in coated particles of a desired size, shape, and quality.

Pyrolytic carbon can be produced and coated onto a substrate surface by known methods. Generally, hydrocarbons and alloying gases are decomposed to prepare a pyrolytic carbon coating on a particle substrate. The particle substrates are included with the hydrocarbons and alloying gases in a fluidized or floating bed at a temperature sufficient to cause deposition of pyrolyzed carbon onto the substrate surface, typically from about 1200 to 1500°. Inert gas flow is used to float the bed of particle substrates, optionally including an inert mixing media. The hydrocarbon pyrolysis results in a high carbon, low hydrogen content carbon material being deposited as a solid material onto the particle substrates.

Alternatively, a carbon coating (sometimes referred to as "ultra-low-temperature isotropic carbon") may be applied to a particle substrate using any one of other various coating processes for depositing carbon, such as a vacuum vapor deposition process. Such a method uses ion beams generated from any of a variety of known processes, such as the disassociation of CO₂, reactive dissociation in vacuum of a hydrocarbon as a result of a glow discharge, sublimation of a solid graphite source, or cathode sputtering of a graphite source. Gold has been found to be an especially suitable substrate material for vacuum vapor deposited carbon. Other substrates,

including but not limited to nickel, silver, stainless steel, or titanium are also quite acceptable as a substrate material for this type of coating process.

The high strength, resistance to breakdown or corrosion, and durability of a carbon surface ensures effective, long term functioning of microparticles in tissue marking applications. The established biocompatibility of carbons such as pyrolytic and vitreous carbon makes the described particles particularly suitable for tissue marking applications. The microparticle substrates may be completely encased by a carbon surface. This results in a smooth coated particle with no substrate exposure on the surface of the particle. Preferred carbon coatings can be in the range of fractions of thousandths of an inch, e.g., about one half of a thousands of an inch (0.0005 inches), on average, covering the surface of the particle substrate.

The microparticles, whether coated or uncoated, are preferably generally rounded particles that have a smooth surface. The smooth surface enhances passage of the microparticles through a hypodermic needle. Microparticles are preferably subjected to a cleaning and sieving process to remove contaminants and to separate out particles of a size less than or greater than a desired size range. The particles may preferably range in size from 100 microns to 1,000 microns in average, transverse cross-sectional dimension, preferably in the range from about 200 to 500 microns, and a particularly preferred size range for use in tissue marking applications can be between about 251 and about 300 microns. To achieve this most preferred such size range, microparticles may be segregated to a selected size range, for example using a sieving process such that the minimum microparticle dimension will pass through a U.S. No. 50 Screen Mesh (300 micron grid size opening) but will not pass through a U.S. No. 60 Screen Mesh (250 micron grid size). That minimum dimension will be the transverse, cross-sectional dimension on oblong or elongated particles, with that dimension coinciding with the particle diameter on generally spherical particles.

Microparticles can be delivered to a tissue site using any instrument or apparatus that can be used to inject an amount of microparticles, preferably contained or suspended in a carrier, through the skin, mucosa, or through an incision in the skin, to a desired tissue site. Preferred instruments include instruments such as hypodermic needles or other similar needle-like apparatuses, such as any small bore instrument, cannula, etc. (All of these types of instruments will be referred to collectively herein,

for convenience, using the term "hypodermic needle" or "needle.") The particular instrument used for delivery is not critical, provided that its components are compatible with the tissue marking composition.

According to one example of a method of delivering microparticles for tissue marking, microparticles can be delivered using a hypodermic needle and a syringe, by inserting the hypodermic needle into a desired tissue site, followed by delivery of the microparticles to the tissue site.

Optionally, any of a variety of surgical or non-invasive or minimally-invasive surgical instruments can also be used to assist in delivery. For example, following removal of a polyp from a colon or a rectum, by known surgical methods, a hypodermic needle can be inserted through the mucosa at the site of the polyp to deliver microparticles. As another example, a mammotome inserted through a small incision in the skin can be maintained in the operative position and a needle can be inserted through its sheath, to precisely deliver microparticles to the site of biopsy.

Once a needle is placed, microparticles can be slowly injected through the needle to the desired tissue site. The microparticles are of a size that can be effectively deposited through a hypodermic needle or like instrument, and that will substantially remain at the tissue site where delivered. If the particles are too small, they can be engulfed by the body's white cells (phagocytes) and carried to distant organs or be carried away in the microvasculature and travel until they reach a site of sufficient constriction to prevent further movement. On the other hand, particles should not be so large that they cannot be effectively delivered using a hypodermic needle or the like. For the method of the present invention, a particularly preferred, average microparticle size can be from about 100 to 1000 microns, alternatively from about 200 to 500 microns or from about 251 to 300 microns, because such sizes can allow injection through small bore instruments and are small enough to avoid migration of the microparticles from the injection site.

The use of microparticles in tissue marking methods, preferably injected by use of a needle and syringe or a like instrument, has advantages over the use of other tissue marking methods. For instance, delivery of microparticles using a needle and syringe allows very precise delivery of microparticles to a desired tissue site. This is particularly true if the biopsy probe used to perform a biopsy is used to delivery the

microparticles, without first moving the probe sheath. Other advantages are that microparticles can be used where other types of tissue markers either cannot be, or are not used. Specifically, tissue-marking clips are not used in the colon or rectum, but microparticles can be injected to these tissues. Additionally, tissue marking clips can sometimes be inadvertently attached to tissue that will move and cause movement of the clip, such as a ligament. The injection of microparticles avoids such problems.

The amount of microparticles introduced in a tissue marking procedure can be any amount sufficient to mark a location to be detected at a later time. The amount delivered can vary depending on factors such as the size of the microparticles the amount of detectable component in the microparticles factors relating to the patient, etc. Such factors will be within the skill of an artisan of ordinary skill in the medical or tissue marking arts, and such an artisan will be able to understand what is a useful amount of microparticles for delivery to body tissue sites.

According to the invention, the microparticles can be contained and used for delivery in a tissue marking composition comprising an injectable combination of microparticles in a biocompatible carrier. The carrier can be any biocompatible fluid capable of delivering the microparticles to a desired tissue site. A carrier may include, a biologically compatible suspension, solution, or other form of a fluid or gel. Examples of materials useful in biologically compatible carriers include saline, dextrans, glycerol, polyethylene glycol, corn oil or safflower oil, other polysaccharides or biocompatible polymers, methyl cellulose, glucan, agarose, either singly or in combination.

The carrier can preferably be an aqueous suspension or solution, other fluid, or gel of polymeric chains of B-D glucose, commonly referred to as β -glucan. The glucose units are linked to each other at the 1-3, 1-4, or 1-6 positions and form polymeric chains ranging to several thousand daltons in weight.

β -glucan is a naturally occurring constituent of cell walls in essentially all living systems including plants, yeast, bacteria, and mammalian systems. Its effects and modulating actions on living systems have been reported by Abel et. al., "Stimulation of Human Monocyte B-glucan Receptors by Glucan Particles Induces Production of TNF- α and IL-B," Int. J. Immunopharmacol., 14(8):1363-1373, 1992. β -glucan, when administered in experimental studies, elicits and augments host

defense mechanisms including the steps required to promote healing by first intent, thereby stimulating the reparative processes in the host system. β -glucan is removed from tissue sites through macrophagic phagocytosis or by enzymatic destruction by serous enzymes. The destruction or removal of β -glucan, as well as its available viscosity and lubricous nature, make it a useful carrier for the microparticles in tissue marking applications.

Aqueous solutions, suspension, fluids, or gels of β -glucan can be produced that have favorable physical characteristics as a carrier for microparticles in tissue marking applications. The viscosity can vary from a thin liquid to a firm, self-supporting gel. Irrespective of viscosity, the β -glucan has excellent lubricity, thereby creating a particle-carrier composition which is easily administered by delivery to a predetermined body site through a small bore needle. A useful β -glucan composition is β -D-glucan containing 4-0-linked- β -D-glycopyranosyl units and 3-0-linked- β -D-glycopyranosyl units. The carrier can be of sufficient viscosity to assure that the microparticles remain suspended therein, for a sufficient time duration to accomplish the injection procedure.

Another example of a carrier material is methyl cellulose or another linear unbranched polysaccharide. Further examples of appropriate carrier materials include agarose, hyaluronic acid, polyvinyl pyrrolidone or a hydrogel derivative thereof, dextran or a hydrogel derivative thereof, glycerol, polyethylene glycol, succinylated collagen, liquid collagen, oil-based emulsions such as corn or safflower, or other polysaccharides or biocompatible organic polymers either singly or in combination with one or more of the above-referenced solutions.

The amount of microparticles to carrier in a tissue marking composition can be any amount that will provide a tissue marking composition that is flowable and injectable, and that will allow a desired amount of microparticles to be delivered to a tissue site. Amounts of microparticles in a tissue marking composition can be in the range from about 20 to 60 percent by volume or from about 25 to 40 percent by volume.

In use, the tissue marking composition can typically be injected as a slurry, suspension, or emulsion, through a needle, into a body tissue site. When deposited into a body tissue, the carrier will be carried away into the body and disperse or be

destroyed. It is necessary that at least some of the microparticles, preferably most or substantially all of the microparticles, are substantially immobile upon delivery to a tissue site for marking. Microparticles used for tissue marking according to the invention are sufficiently immobile to be used for tissue marking applications. If the microparticles tend to move at all after delivery to a tissue site, the microparticles generally will do so only up the path of the needle used to inject them.

While subsequent portions of the description include language relating specifically to tissue marking in breast biopsy applications, all types of tissue marking applications are considered to be within the contemplation of the present invention. Examples include other types of biopsy applications such as colon, rectum, or prostate biopsies, and non-biopsy applications including tissue marking for other types of medical procedures. The tissue may also be marked to provide a target at which a beam of radiation can be precisely directed. One of ordinary skill in the medical or biopsy arts will understand and appreciate how detectable microparticles can be used in these and other tissue marking or biopsy processes.

Factors that might be considered, controlled, or adjusted for in applying the process to a particular tissue marking application might include consideration of the composition of the microparticles; the amount of microparticles to be delivered to the body site; factors relating to the method of delivery including the particular equipment (a needle or biopsy probe) used for delivery, and the method and route used to place the dispensing end of the delivery device at the desired body site. All of these factors will be appreciated by one of ordinary skill in the tissue marking or medical arts, and can be readily dealt with to apply the described methods to a wide variety of tissue marking procedures.

Biopsy is a method by which a tissue sample is removed from a site of a body to diagnose the tissue as healthy or diseased. Biopsies are performed on tissues of many different body organs, including prostate and breast tissues. The means used to perform the biopsy can include any equipment and techniques generally known or useful in biopsy procedures.

Breast biopsies can be performed using stereotactic, vacuum-assisted breast biopsy techniques and equipment therefore. Such techniques and equipment involve the use of minimally invasive instruments and techniques such as automated surgical

biopsy devices. These methods and devices relate to percutaneous procedures that include inserting a needle-like instrument through a very small incision in the breast to access the tissue mass of interest and obtain a tissue sample for later examination and analysis. See United States Patent 6,086,544. It is not uncommon in modern breast
5 biopsies using a mammotone breast biopsy system sold under the trade name BIOPSYS, from Ethicon Endo-Surgery, Inc., for all evidence of a lesion to be removed during the biopsy.

Detectable microparticles can be used to mark the site of a breast biopsy. The microparticles can be used to locate the biopsy site in case malignancy is determined,
10 thereby enabling a return to the site and subsequent surgical treatment even if the mammographic findings associated with the original lesions were removed completely.

The detectable microparticles can be delivered to the site of the breast biopsy using any sort of a needle delivery system. According to a preferred method of the invention, microparticles can be delivered using the same biopsy equipment, as used to
15 perform the biopsy, so as to achieve very precise marking of the biopsied site.

Examples

A rabbit research study was conducted to evaluate applications of radiopaque pyrolytic carbon coated zirconium oxide beads. Rabbits were selected because
20 auricular injection has frequently been used to quantify the persistence of materials, the cartilage in the ear provides a substrate over which the material can be inserted and subsequently measured. Migration from the insertion site proximally can be readily observed and the relative constancy of the landmarks in a smaller animal allows bony landmarks for dissection at necropsy.

25 The carbon coated beads were injected into rabbits in ear subcutaneous tissue, and stomach/esophagus sub-mucosa. A total of 5 rabbits had injections (0.5 – 1.0 ml) in both ears and 2 rabbits had injections (0.25 ml each) in the intestine/stomach location.

30 Radiopaque carbon coated beads were injected subcutaneously between the skin and the auricular cartilage on the convex surface of ears, and subs-serosally along the greater curvature of the stomach in rabbits. Weekly examination demonstrated

minimal inflammatory response to the injected area. At 5 weeks (ear) and 8 weeks (stomach/esophagus) post-procedure the radiopaque beads appeared stable in place.

Persistence in location was measured by radiography and necropsy. Flat film radiographs were taken pre-and post-procedure to document persistence and non-migration. Radiographs were taken at sacrifice to document non-migration.

Ear Procedure

Volumes between 0.5 – 1.0 ml of beads were injected in both ears of 5 rabbits. The beads were injected subcutaneously between the skin and the auricular cartilage on the convex surface of the ear using disposable 16-gauge needles. All ears were examined after injection and a x-ray of the ear was taken for record purposes.

1 Week Post-procedure

There were no evidence of swelling, inflammation or edema associated with the injection site or the injections.

2 Weeks Post-procedure

Minor reddish and swelling around each injected bulk was observed.

3 Weeks Post-procedure

All injection sites were easily visible with little evidence of redness or swelling.

5 Weeks Post-procedure

At 5 weeks post-procedure, rabbits #1 and #2 were necropsied.

The ears as well as the internal organs were examined grossly. Upon dissection of the periauricular tissue on the skull and anterior and posterior to the base of the ear, no beads were observed in the tissues.

The ears were examined for migration of beads from the site of injection. The examinations were accomplished by illuminating the tissues with bright light. No particles were observed beyond the boundaries of the lesions.

Stomach/Esophagus Procedure

Using the gastric model, multiple sub-serosal injections were made along the greater curvature of the stomach. Approximately 0.25 ml of material was injected at each of 4 sites in one rabbit and 3 sites in another rabbit. The injections were made using a 1-ml syringe of material and a 16-gauge needle.

2 Weeks Post-procedure

Observations were consistent with that of the ear data.

8 Week Post-Procedure

5 At 8 weeks post-procedure, following radiography, both rabbits were necropsied.

10 There was no apparent loss of beads or migration of beads from the injection site. No gross migration of particles on the surface of the stomach or on the surface of the abdominal structures was noted. The injection sites appeared well healed with no evidence of erythema or swelling noted in or about the injection sites. Gross examination of the abdominal structures did not demonstrate any gross abnormalities.

What is claimed:

1. A method for tissue marking comprising delivering detectable microparticles to a tissue site and detecting the microparticles.

2. The method of claim 1 wherein the microparticles comprise one or more radiopaque materials.

3. The method of claim 2 wherein the radiopaque material is titanium, aluminum oxide, zirconium oxide, or a mixture thereof.

4. The method of claim 1 wherein the microparticles comprise a substrate and radiopaque coating.

5. The method of claim 1 wherein the microparticles comprise a radiopaque substrate and a coating.

6. The method of claim 1 wherein the average, transverse cross-sectional dimension of the microparticles is between 100 and 1000 micrometers.

7. The method of claim 1 wherein the average, transverse cross-sectional dimension of the microparticles is between 251 and 300 micrometers.

8. The method of claim 1 wherein the microparticles are carried in a biocompatible carrier to carry and deliver the microparticles.

9. The method of claim 1 wherein the method comprises injecting the microparticles using a hypodermic needle.

10. The method of claim 1 wherein the method comprises detecting the microparticles using radiography or fluoroscopy.

11. A method for marking a biopsy site, the method comprising:
- performing a biopsy; and
 - marking the site of the biopsy using detectable microparticles injected through a hypodermic needle.

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Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i)) for the following designations CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR)
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR)

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(54) Title: TISSUE MARKING USING BIOCOMPATIBLE MICROPARTICLES

(57) Abstract: Described are methods of tissue marking using microparticles that have carbon surfaces, and that contain a contrast agent. Preferred microparticles include a permanently radiopaque particle substrate and a pyrolytic carbon surface.

INTERNATIONAL SEARCH REPORT

national Application No

PCT/US 01/25352

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K49/04 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EMBASE, BIOSIS, EPO-Internal, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 38579 A (BUSH MARY ELIZABETH ;CONSTON STANLEY R (US); FAWZI NATALIE V (US);) 6 July 2000 (2000-07-06) claims	1,11
X	EL-GARHY, M. ET AL: "Pharmaceutical studies on a technetium-99m-coated carbon suspension intended for diagnostic pulmonary scanning" STRAHLENTHERAPIE (1971), 142(4), 463-6 , XP008006856 tables 1,2	1,2,4-9

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

19 August 2002

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27/08/2002

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INTERNATIONAL SEARCH REPORT

Information on patent family members

ational Application No

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0038579	A	06-07-2000	US 6371904 B1	16-04-2002
			US 6356782 B1	12-03-2002
			AU 2592400 A	31-07-2000
			EP 1152696 A2	14-11-2001
			WO 0038579 A2	06-07-2000
			US 2002107437 A1	08-08-2002
			US 2002035324 A1	21-03-2002
WO 0200192	A	03-01-2002	US 6355275 B1	12-03-2002
			WO 0200192 A2	03-01-2002

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/25352

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>LI C ET AL: "Biodistribution of cyclic carbonate of ioxilan: a radiopaque particulate macrophage imaging agent." ACADEMIC RADIOLOGY, (1996 JUN) 3 (6) 500-6., XP008006857 figure 1</p>	1-10
E	<p>WO 02 00192 A (CARBON MEDICAL TECHNOLOGIES IN) 3 January 2002 (2002-01-03) cited in the application claims</p>	1-10

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/25352

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-11 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.